TXTL cell culture Protocol

1. 2xYT-P medium and agar plate supplemented with suitable antibiotic is prepared.
2. Streak *E. coli* BL21 Rosetta – 80C glycerol stock on the 2xYT-P agar plate, incubation 37C overnight
3. Pick from the BL21 agar plate and grow in 10ml 2xYT medium in 37C for 8 hours, this is start culture 1.
4. After 8 hours, dilute 1ml of start culture 1 1:100 in fresh 2xYT-P medium in a 500ml conical flask. Grow the bacterial culture in 37C overnight. This is start culture 2
5. The next day morning dilute 2.5ml of start culture 2 in 1:100 in 2xYT-P medium without antibiotics. Grow in 37C until OD reaches 1.5.
6. Place bacterial culture flask in ice bath for 10 minutes, pellet down the cell by centrifuging in 5000xg, 4C, for 12 minutes. Discard the supernatant.
7. Wash the cell using 100ml S30A buffer by vigorous shaking to resuspend the pellet.
8. Pellet down the cell by centrifuging in 5000xg, 4C, for 12 minutes. Discard the supernatant.
9. Repeat step 7-8
10. Weight the pellet and add appropriate volume of S30A buffer to resuspend the pellet.
11. Lyse the cell using Constant cell disruption system using 25 K psi.
12. Collect the cell lysate in 1.5 mL micro centrifuge tube and centrifuge at 13000xg 4C for 10 minutes,
13. Collect clear supernatant in 1.5mL micro centrifuge tube and incubate in 37 C for 1.5 hours.
14. Centrifuge cell lysate at 13000xg 4C for 10 minutes.
15. Collect clear supernatant in 15mL tissue culture tube, and invert several times to homogenize the lysate.
16. Dialyze the processed cell lysate against S30B buffer in 4C for 3 hours.
17. Aliquot dialyzed cell lysate in 30ul per 1.5mL micro centrifuge tube.
18. Flash freeze the aliquot.

Buffer Recipe

2xYT+P+Cm agar plate: Prepare 1.24 g 2xYT, 1.6 ml potassium phosphate dibasic solution @ 1 M, 0.88 ml potassium phosphate monobasic solution @ 1 M, 0.6 g agar, and water to 40 ml. Autoclave. Let cool to 50 °C and add 40 µl Cm. Aliquot 25 ml into

a 100 x 15 mm Petri dish, and let cool for an hour.
2xYT+P media: Prepare 124 g 2xYT, 160 ml potassium phosphate dibasic solution @1 M, 88 ml potassium phosphate monobasic solution @ 1M, and water to 4 L. Aliquot out into 2 x 1.88 L and 0.24 L. Autoclave.

S30A buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, 50 ml Tris at 2M, acetic acid (to pH 7.7), and water to 2 L. Autoclave, store at 4 °C, add 4 ml 1 M DTT before use.

S30B buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, Tris at 2 M (to pH 8.2), and water to 2 L. Autoclave, store at 4 °C, add 2 ml 1 M DTT before use.